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The structure of the oligosaccharides of $\alpha_3\beta_1$ integrin from human ureter epithelium (HCV29) cell line✪

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There is a growing line of evidence that glycosylation of α and β subunits is important for the function of integrins. Integrin $\alpha_3\beta_1$, from human ureter epithelium cell — line HCV29, was isolated by affinity chromatography on laminin GD6 peptide. Characterization of its carbohydrate moieties was carried out using sodium dodecyl sulfate/polyacrylamide gel electrophoresis followed by Western blotting on Immobilon P and on-blot deglycosylation with peptide *N*-glycosidase-F. Profiles of N-glycans for each subunit were obtained by matrix-assisted laser desorption/ionization mass spectrometry. Our findings demonstrated, in both subunits of integrin $\alpha_3\beta_1$, the presence of complex type oligosaccharides with a wide heterogeneity. Bi- tri- and tetra-antennary structures were the most common, while high-mannose type structures were minor. Also the presence of short poly-*N*-acetyllactosamine entities was shown. These results show that while the predominant oligosaccharides of both subunits are identical, some slight differences between them do exist.

Adhesive interactions between cells and extracellular matrix (ECM) proteins play a critical role in numerous biological processes such as cell proliferation, migration and differentiation (Petruzzelli *et al.,* 1999). The loss of adhesive interactions as well as a stimula-

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Abbreviations: ECM, extracellular matrix; mAbs, monoclonal antibodies; DSA, *Datura stramonium* agglutinin; GNA, *Galantus nivalis* agglutinin; MALDI, matrix-assisted laser desorption/ionization; PHA-L, *Phaseolus vulgaris* agglutinin; PNGase-F, peptide *N*-glycosidase; RGD, arginine-glycine-aspartic acid tripeptide.

tion of adhesion may result in a disease state. Cell adhesion is mainly mediated by integrins, the heterodimeric transmembrane glycoproteins that mediate cell : cell and cell : ECM attachment and link the extracellular matrix to the actin cytoskeleton (Schoenwaelder & Burridge, 1999). Binding to their ligands: fibronectin, vitronectin, collagen and laminin leads to subsequent clustering and activation of integrins followed by a series of intracellular responses including changes in protein phosphorylation, intracellular calcium level and activation of the mitogen activated protein kinase cascade (Turner, 2000). The binding specificity of the integrin extracellular domain is largely determined by the conformation of the individual α and β subunits which comprise the complex (Belkin & Stepp, 2000).

Integrin α β is particularly interesting because of its role in development, wound healing and tumorigenesis. Its function is more complex than that of a simple adhesion receptor or even a signalling molecule. In many instances it appears to modulate events that are primarily mediated by other adhesion molecules (Kreidberg, 2000). $\alpha_3\beta_1$ integrin adhesion functions are remarkably versatile. It interacts with multiple matrix ligands apparently by both sequence Arg-Gly-Asp (RGD)-dependent and RGD-independent mechanisms, possessing functionally distinct ligand binding motifs (Elices *et al.,* 1991). In epithelial cells it acts as a receptor for the basement membrane, while in neuronal and tumor cells it mediates migration (Kreidberg, 2000). The $\alpha_3\beta_1$ integrin has been reported to be a receptor for laminin (Gehlsen *et al.,* 1992; Belkin & Stepp, 2000), fibronectin (Tawil *et al.,* 1996) and collagen (Elices *et al.,* 1991). Recent studies have shown that laminin-5, laminin-10 and laminin-11 are preferred ligands for integrin $\alpha_3\beta_1$ (Fukushima *et al.,* 1998). However, due to conflicting reports obtained using function-blocking antibodies and different cell lines, it seems that $\alpha_3\beta_1$ function is cell- and tissue-dependent (Belkin & Stepp, 2000). Interestingly, the $\alpha_3\beta_1$ integrin has been shown to be a transdominant inhibitor of other integrins in mouse keratinocytes and to downregulate actin stress fibres and focal adhesion (Hodivala-Dilke *et al.,* 1998).

Oligosaccharides play a vital role in normal growth and development of living organisms (Varki, 1993). They influence the three-dimensional structure and function of glycoproteins (Kobata, 1992) susceptibility to proteases (Varki, 1993), serve as ligands for lectins (Wada & Makito, 2001) and contribute to cell : cell (Drickamer & Taylor, 1998) and cell : ECM interactions (Morgenthaler *et al.,* 1990). Also, altered expression and/or structure of cell-associated oligosaccharides is one of the most frequent biochemical feature associated with tumorigenesis and metastasis.

The exact function of integrin oligosaccharides remains to be determined. However, it has been proposed that N-linked oligosaccharides may be important for integrin receptor assembly and ligand binding (Chammas *et al.,* 1993; Zheng *et al.,* 1994). In light of these studies we performed a preliminary assay of $\alpha_3\beta_1$ integrin glycans in different bladder cell lines (Lityńska *et al.,* 2000). These studies showed that in epithelium cell line (HCV29) cells the α_3 subunit reacted only with *Galantus nivalis* agglutinin (GNA) – the lectin specific for high mannose type glycans while β_1 subunit reacted only with lectins recognising sialic acid. However, the lack of reaction with other lectins could be caused not only by the absence of other structures but also could be due to a low amount of this integrin in the material analysed, therefore, we decided to perform a detailed oligosaccharide analysis of integrin $\alpha_3\beta_1$ isolated from human ureter HCV29 line.

MATERIALS AND METHODS

Cell line and culture conditions. The cell line of non-malignant transitional epithelial cell bladder HCV29 was obtained from the

Cell Line Collection of the Institute of Immunology and Experimental Therapy, Polish Academy of Sciences (Wrocław, Poland). The cells were maintained in RPMI 1640 medium (Sigma) supplemented with 10% fetal calf serum (Boehringer), 100 units/ml of penicillin and 100 μg/ml of streptomycin. After reaching confluence the cells were washed twice and then harvested in phosphate buffered saline. The cell pellets were homogenized in 50 mM Tris/HCl containing 15 mM NaCl, 1 mM $MgCl_2$, 1 mM $MnCl_2$, pH 7.4, and protease inhibitor cocktail (Sigma P-2714) by sonication (five times 5 s each) (Bandelin electronic UW 70 Sonopuls Gm 70), extracted for 1 h on ice with the same buffer containing additionally 30 mM octyl-β-D-glucopyranoside and 3% protamine sulfate. Finally, the cell extracts were clarified by centrifugation at 35 000 × ***g*** for 1 h (L7-65, Beckman).

Affinity chromatography. GD6 peptide (residues 3011–3032 of the globular domain of the laminin A chain) (Gehlsen *et al.,* 1992) was synthesised at the Faculty of Chemistry, University of Gdańsk (Poland). This peptide was coupled to activated CH-Sepharose (Sigma) according to the manufacturer's instruction (Pharmacia Fine Chemicals AB). Briefly, 10 mg of HPLC-purified peptide GD6 was dissolved in 0.5 ml of coupling buffer (0.1 M $NaHCO_3$, pH 8.0), added to 3 ml of preswollen beads and agitated overnight at 4°C. Unbound peptide was removed by washing the beads with the coupling buffer and the remaining reactive groups were blocked by treatment with a Tris/HCl buffer (0.1 M, pH 8.0) for 1 h. The product was washed alternately with high and low pH buffer solutions four times. The peptide-Sepharose beads were then packed into a column and stored in extraction buffer in the presence of a bacteriostatic agent at 4–8°C.

The cleared cell lysate (about 5 mg of protein) was then loaded onto the column pre-equilibrated with extraction buffer and was allowed to interact with the immobilised ligand overnight at 4°C. The column was extensively washed with extraction buffer, and the bound material was eluted with 20 mM EDTA in 50 mM Tris/HCl, pH 7.4, containing 30 mM octyl-β-D-glucopyranoside. The column was subsequently eluted with 0.5 and 1 M NaCl to remove any remaining bound proteins. Fractions eluted with EDTA and NaCl (10 ml each) were collected, dialysed against water and concentrated to 0.5 ml by lyophilisation. In order to remove detergent from the sample, Bio-Beads SM-2 (Bio-Rad) were used. The sample was laid on the Bio-Beads equilibrated with 10 mM Tris/HCl, pH 7.4 (1 g of beads per 0.07 g of detergent), washed with the above buffer at 0.03 ml/min and fractions of 1 ml were collected, pooled, concentrated in VIVA SPIN 50 and then analysed by SDS/PAGE under nonreducing conditions and probed with specific antibodies.

SDS/PAGE and immunodetection of α_3 and β_1 subunits. For deglycosylation of the α_3 and β_1 subunits prior to SDS/PAGE, 3 μg of the material eluted from GD6-Sepharose column was denatured by heating at 100°C for 2 min in 5 μl of buffer (20 mM sodium phosphate, pH 7.5, containing 50 mM EDTA, 0.5% SDS, 5% mercaptoethanol). After cooling, 1 μl of 10% non-ionic detergent (Nonidet P40) followed by 0.35 U PNGase-F (Boehringer) were added and the solution was incubated at 37°C overnight. Material eluted from GD6-Sepharose before or after deglycosylation was separated by 8% SDS/PAGE in nonreducing condition according to Laemmli (1970) and transferred to a PVDF membrane, by electrophoretic blotting (Towbin *et al.,* 1979) for 1.5 h at 100 V. The efficiency of protein transfer was at least 95% as checked by staining the gel with Coomassie Brilliant Blue R-250. Proteins immobilised on PVDF membranes were stained with Ponceau S followed by destaining in H_2O. The blots were blocked in TBS/Tween (0.02 M Tris/HCl, pH 7.6, containing 0.15 M NaCl and 0.1% Tween 20), with 1% bovine serum albumin (BSA). Afterwards, the membranes were sequentially incubated with specific antibodies diluted in TBS/Tween

with 1% BSA (1:1000 for β_1 subunit and 1:100 for α_3 subunit) for 2 h and 18 h, respectively. Mouse monoclonal antibodies to human integrin subunit β_1 (clone B3B11) from Chemicon, and rabbit polyclonal antibodies to human α_3 subunit (Chemicon) were used. After triple wash with TBS/Tween the blots were incubated with alkaline phosphatase coupled to secondary goat anti-mouse or anti-rabbit Ig (Boehringer) (1:500 in TBS/Tween with 1% BSA) for 1 h and the bands were localised with 4-nitro blue tetrazolium chloride as a substrate.

Glycan chain analysis. Individual protein bands corresponding to the α_3 and β_1 subunits were excised from the Immobilon P membrane and further glycan analysis was carried out according to the method described by Kuster *et al.* (1997) as modified by Hoja-Łukowicz *et al.* (2000) or was performed with the use of Glycan Differentiation Kit (Boehringer) according to Haselbeck *et al.* (1990) as described in details earlier (Lityńska & Przybyło, 1998).

Protein alkylation. The excised Immobilon P pieces were placed into Eppendorf tubes and washed twice with 1 ml of 20 mM $NaHCO_3$, pH 7.0, for 15 min each. The wash was discarded, dithiothreitol was added and the protein was reduced at 60°C for 30 min. After cooling to room temperature, 20 μl of 100 mM iodoacetamide was added and the protein was alkylated for 30 min at room temperature in the dark. The reducing and alkylation reagents, as well as residual SDS, were then removed by incubation in 1/1 acetonitrile/fresh 20 mM $NaHCO_3$, pH 7.0, for 60 h. Subsequently, the membrane pieces were incubated in blocking solution (Boehringer).

In situ *digestion.* Prior to deglycosylation Immobilon P pieces were washed three times with 20 mM $NaHCO_3$, pH 7.0, for 15 min each. The washings were discarded and replaced with 3 U of PNGase-F in 30 μl of 20 mM $NaHCO_3$, pH 7.0, and incubated at 37°C for 12–16 h (Tarentino *et al.,* 1985).

Sugar extraction. After deglycosylation the incubation buffer, was completely dried in a SpeedVac^R Plus (Savant), and the residue dissolved in 10 μl of ultrapure water (Milli-Q Plus, Millipore, Bedford, CA, U.S.A.) was applied to microcolumn clean-up.

Microcolumn clean-up of sugars. Prior to MALDI MS, a microcolumn consisting of an Eppendorf GELoader pipette tip packed with about 5 μl each of AG-3 (OH^- form, bottom) and AG-50 (H^+ form, top) was used (Kuster *et al.,* 1997). The column was washed with 100 μl of water and an aliquot of the sugar sample was applied. Glycans were eluted with 100 μl of water and dried in a SpeedVac.

MALDI mass spectrometry. Matrix-assisted laser desorption ionization (MALDI) mass spectra were recorded by using a Voyager DE-PRO MALDI-TOF mass spectrometer (Applied Biosystem) equipped with a delayed extraction mode. The MALDI matrices were prepared by dissolving 25 mg of 2,5-dihydroxybenzoic acid in 1 μl of acetonitrile/0.2% trifluoroacetic acid (70:30, v/v). Typically, 1 μl of the analyte was then added. Mass calibration was performed with the MH^+ ion of insulin set at *m/z* 5734.6 and a known peptide ion at *m/z* 1209.7. Raw data were analysed using the computer software provided by the manufacturer and are reported as average masses.

Other methods. Protein content was determined by the dye-binding method (Bradford, 1976) using bovine serum albumin as a standard.

RESULTS

In order to isolate the $\alpha\ \beta$ integrin from human ureter epithelium (HCV29) cell line we used affinity chromatography on GD6 peptide followed by ultrafiltration in VIVA SPIN 50 MW. Electrophoretic analysis of the resulting preparation revealed that the major species consists of the α_3 (158 kDa) and β_1 subunits

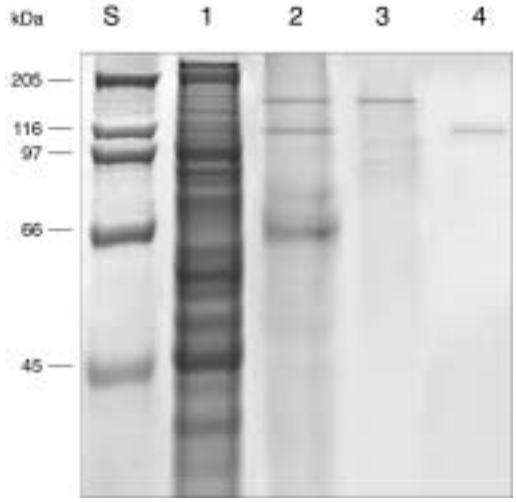

Figure 1. SDS/PAGE of cell lysate and GD6 peptide bound $\alpha_3\beta_1$ integrin from human ureter epithelium.

The cell lysate and GD6 peptide bound material were separated by SDS/PAGE and stained with Coomassie Brilliant Blue or analysed by Western blotting with antibodies specific for α or β_1 subunit. Lane S – molecular mass markers (HMW, Sigma), lane 1 – cell lysate (50 μg), lane 2 – GD6 peptide bound material (30 μg), lane 3 – α_3 subunit (3 μg), lane 4 – β_1 subunit (3 μg).

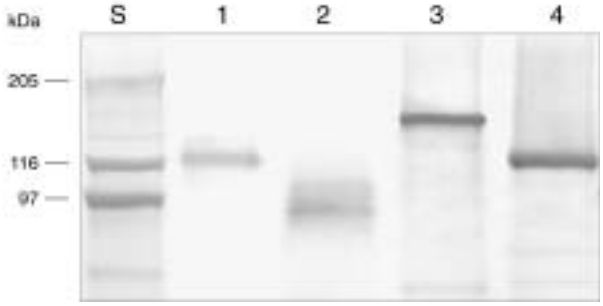

Figure 2. SDS/PAGE of $\alpha_3\beta_1$ integrin treated with PNGase-F.

GD6 peptide purified α β_1 integrin (3 μg) was digested with PNGase-F, separated by SDS/PAGE and analysed by Western blotting with specific antibodies for α_3 or β_1 subunit. Lane S – molecular mass markers (HMW, Sigma), lane 1 – β_1 subunit, lane 2 – β_1 subunit treated with PNGase-F, lane 3 – α_3 subunit, lane 4 – α_3 subunit treated with PNGase-F.

(116 kDa), however, the presence of smaller proteins of 79, 67, 54 and 49 kDa was also noted (Fig. 1).

Treatment of the $\alpha_3\beta_1$ integrin with PNGase-F decreased the molecular mass of α_3 and β_1 chains by 42 and 18 kDa, respectively (Fig. 2) suggesting extensive glycosylation of both subunits.

Positive ion spectra were acquired by MALDI MS for the glycans released from the α_3 (Fig. 3, Table 1) and β_1 (Fig. 4, Table 2) subunits. The signals at *m/z* 1419.9 and 1743.7 as well as at 1580.1 and 1743.7 likely originate from high-mannose type glycans of the α_3 and β_1 subunits, respectively. The *m/z* values of 1505.7, 1975.5 and 2122.1 in both subunits correspond to biantennary structures with (*m/z* 2122.1) or without fucose residues. The signals at *m/z* 2174.7, 2487.5, 2539.7 and 2852.1 may be due to tri- and tetraantennary entities, while the signal at *m/z* 2903.5 likely originates from a polylactosamine structure. The signal at *m/z* of 1905.5 could correspond to a tetraantennary glycan or alternatively to a biantennary one with a polylactosaminyl residue. In general, both subunits had a similar pattern of glycosylation. The only exception were the signals at *m/z* 3168.7 characteristic exclusively for the β_1 subunit and at *m/z* 2642.3 and 2903.5 for α_3.

The presence of short poly-*N*-acetyllactosamine units and β1-6 branched oligosaccharides was further confirmed by lectin blotting carried out with *Datura stramonium* agglutinin (DSA) and *Phaseolus vulgaris* agglutinin (PHA-L). Both the α_3 and β_1 subunits reacted with DSA and PHA-L, but with different intensity. More intensive staining with both lectins was associated with the β_1 subunit (Fig. 5).

DISCUSSION

Integrin $\alpha_3\beta_1$ has never been purified in amounts sufficient for definitive analysis. The classical methods did not yield a pure preparation of soluble integrin $\alpha_3\beta_1$. In contrast, immunopurification on beads coated with anti α_3 integrin mAbs takes place under crude conditions of elution that are at least partly dena-

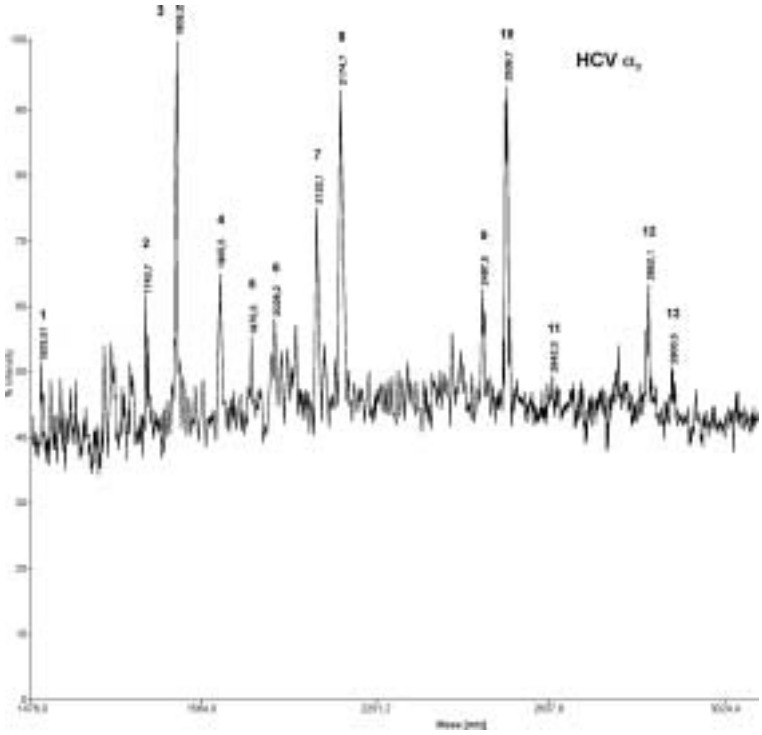

Figure 3. Positive MALDI MS mass spectra of oligosaccharides obtained by PNGase-F digestion of α_3 subunit.

Table 1. Hypothetical oligosaccharide structures observed in MALDI MS spectra of α_3 subunit.

■, *N*-acetyl-D-glucosamine; ❍, D-mannose; ●, D-galactose; ▲, neuraminic acid; ★, L-fucose.

Peak	Mass	Hyphothetical Structure	
1	1503.7	$Hex_4HexNAc_4$	
2	1743.7	$Hex_8HexNAc_2$	
3	1809.2	$Hex_5HexNAc_4Fuc$	
4	1905.5	$Hex_4HexNAc_6$	or
5	1975.5(MNa^+)	$Hex_5HexNAc_4Sia$	
6	2028.2	$Hex_6HexNAc_5$	
7	2122.1(MNa^+)	$Hex_5HexNAc_4FucSia$	
8	2174.7	$Hex_6HexNAc_5Fuc$	or
9	2487.5(MNa^+)	$Hex_6HexNAc_5FucSia$	or
10	2539.7	$Hex_7HexNAc_6Fuc$	
11	2642.3	$Hex_6HexNAc_8$	
12	2852.1(MNa^+)	$Hex_7HexNAc_6FucSia$	
13	2903.5	$Hex_8HexNAc_7Fuc$	

turing (Eble *et al.,* 1998) and can cause a loss of sialic acid residues.

According to Gelhsen *et al.* (1992) a synthetic peptide GD6 derived from the carboxy terminus of the laminin A chain specifically bound the $\alpha_3\beta_1$ integrin, while it did not bind other integrins. The proteins eluted from the GD6 column with EDTA and further analysed by immunoprecipitation using different anti integrin α and β chains specific antibodies showed the presence of the α_3 and β_1 integrin subunits exclusively (Gelhsen *et al.*, 1992). In our preparation, proteins of lower molecular mass were also present. This is not surprising due to the fact that at least fifteen different molecules have been described as either a laminin receptor or laminin-binding proteins (Castronovo, 1993; Belkin & Stepp, 2000). However, the presence of these proteins had no influence on further analysis because the method of Kuster *et al.* (1997) adopted by us (Hoja-Łukowicz *et al.,* 2000) allowed the analysis of a single protein even in whole cell lysate.

Our previous studies carried out on whole cell lysate of HCV29 cells showed that the α_3 subunit reacted only with GNA indicating the presence of high mannose type glycans in α_3

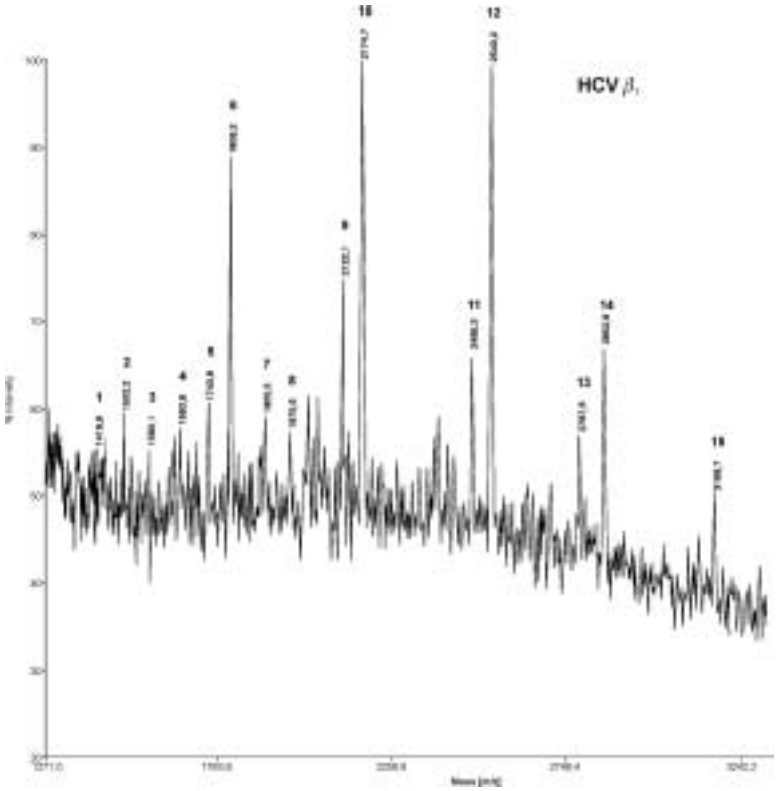

Figure 4. Positive MALDI MS mass spectra of oligosaccharides obtained by PNGase-F digestion of β_1 subunit.

Table. 2. Hypothetical oligosaccharide structures observed in MALDI MS spectra of β_1 subunit.

■, *N*-acetyl- D-glucosamine; ❍, D-mannose; ●, D-galactose; ▲, neuraminic acid; ★, L-fucose.

Peak	Mass	Hyphothetical Structure	
1	1419.9	$Hex_6HexNAc_2$	
2	1502.2	$Hex_4HexNAc_4$	
3	1580.1	$Hex_7HexNAc_2$	
4	1663.8	$Hex_5HexNAc_4$	
5	1743.8	$Hex_8HexNAc_2$	
6	1809.2	$Hex_5HexNAc_4Fuc$	
7	1905.5	$Hex_4HexNAc_6$	
8	1975.6(MNa_2^+)	$Hex_5HexNAc_4Sia$	
9	2122.1(MNa_2^+)	$Hex_5HexNAc_4FucSia$	
10	2174.7	$Hex_6HexNAc_5Fuc$	
11	2485.3(MNa_2^+)	$Hex_6HexNAc_5FucSia$	
12	2540.2	$Hex_7HexNAc_6Fuc$	
13	2787.6	$Hex_8HexNAc_5FucSia$	
14	2852.8(MNa_2^+)	$Hex_7HexNAc_6FucSia$	
15	3168.7(MNa_3^+)	$Hex_7HexNAc_6FucSia_2$	

but not in the β_1 subunit (Lityńska *et al.*, 2000). Our observations were consistent with the results of Elbe *et al.* (1998) who also found only high-mannose type glycans in recombined $\alpha_3\beta_1$ integrin. The purification of integrin $\alpha_3\beta_1$ by affinity chromatography on the GD6 peptide, and using MALDI MS – a much more sensitive method of analysis – allowed us to show that both subunits of the $\alpha_3\beta_1$ integrin from HCV29 cells possessed also bi- tri- and tetraantennary complex type glycans. In addition these glycans can be fucosylated and sialylated. A fraction of the oligosaccharides could be of the poly-*N*-acetyllactosamine type. Thus the present results demonstrate that an analysis of whole cell lysate followed by immunochemical identification may only be treated as a preliminary test. For studies of glycan structure of a chosen cell protein one needs to at least partially purify the protein as was currently done. The results of Elbe *et al.* (1998) should be regarded as very specific since the studies were performed on the $\alpha_3\beta_1$ integrin/Fos or $\alpha_3\beta_1$ integrin/Jun fusion proteins whose glycosylation in the system used – insect cells – is at least controver-

Figure 5. The reaction of GD6 peptide bound $\alpha_3\beta_1$ integrin reaction with lectins.

The GD6 peptide bound material was probed with digoxigenin-labeled *Phaseolus vulgaris* agglutinin (PHA-L) and *Datura stramonium* agglutinin (DSA) lectins. For details see Materials and Methods. Lane S – molecular mass markers (HMW, Sigma), lane 1 – α subunit (immunodetection), lane 2 – detected with PHA-L, lane 3 – detected with DSA, lane 4 – β_1 subunit (immunodetection). For immunodetection 3 μg GD6 peptide bound material was used and for lectins analysis – 15 μg.

sial and therefore may not be representative for a typical integrin glycosylation process taking place within mammalian cells (Marchal *et al.,* 2001).

Prokopishyn *et al.* (1999) demonstrated that $\alpha_3\beta_1$ integrin from colon cancer cells is a sialoglycoprotein carrying $\beta 1-6$ branched oligosaccharides and short poly-*N*-acetyllactosamine units, structures implicated in cancer metastasis. Those data were obtained based on endo-β galactosidase digestion and confirmed by lectin blotting carried out with DSA, which binds short poly-*N*-acetyllactosamine and *N*-acetyllactosamine units in antenna. Both the α_3 and β_1 subunits reacted with DSA, with more intense staining associated with the β_1 subunit. Similarly, blotting with PHA-L indicated that both the α_3 and β_1 subunits contain $\beta 1-6$ branched oligosaccharides, the β_1 subunit again showing more intense staining. Our results well corresponded with those of Prokopishyn *et al.* (1999) indicating different glycosylation of the α_3 and β_1 chains at least in respect to branched oligosaccharides structures. It should be noted that in our spectra the signals at *m/z* 2174.3 and 2485.3 corresponding to $Hex_6HexNAc_5Fuc$ and $Hex_6HexNAc_5FucSia$ can be attributed to triantennary as well as biantennary glycans with short poly-*N*-acetyllactosamine units while the *m/z* of 2903.5 ($Hex_8HexNAc_7Fuc$) without doubt corresponds to a poly-*N*-acetyllactosamine unit.

Based on lectin analysis of whole cell extracts we were not able to notice any O-glycans (negative staining with peanut agglutinin, results not shown). This does not conclusively demonstrate a lack of O-linked oligosaccharides in the $\alpha_3\beta_1$ integrin, but since the focus was on major glycans of this protein – i.e. N-glycans, studies on O-glycosylation were not continued.

It is noteworthy that poly-*N*-acetyllactosamine structures have been detected on a variety of cancer cells. Prokopishyn *et al.* (1999) have shown that $\alpha_3\beta_1$ integrin expressed by human colon carcinoma cells was a major carrier of oncodevelopmental carbohydrate epitopes whose presence may modulate tumor cell adhesion and migration. On the other hand, Li & Roth (1997) unequivocally demonstrated that most cells in normal human organs are reactive with PHA-L. While the presence of $\beta 1-6$ branched oligosaccharides contributes to cancer metastasis by influencing tumor cell adhesion and invasion, the role of PHA-L positive glycoproteins in normal human epithelia is currently unknown. In the opinion of Elbe *et al.* (1998) carbohydrate chains stabilize the elongated structure of the C-terminal portion of the integrin ectodomain. The studies of Zheng *et al.* (1994) clearly demonstrate that N-glycosylation of both the α_5 and β_1 subunits is essential for the association of the subunits and for optimal binding to fibronectin. Although the exact structure of the N-linked oligosaccharide chains of the $\alpha_5\beta_1$ integrin remains to be elucided, some N-linked glycans are essential for the maintenance of the conformation favourable for ligand-binding specificity. GlcNAc linked to core mannosyl structure seems to be essential while sialyl and gala-

ctosyl residues of the N-linked structures are nonessential for the maintenance of $\alpha_5\beta_1$ function (Zheng *et al.,* 1994). On the other hand, Chammas *et al*. (1993) have shown that in adhesion of cells to laminin *via* integrin $\alpha_6\beta_1$, α-galactosyl residues on the integrin α-chain were involved as laminin-binding determinants, while β-chain complex structures were associated with cell spreading. It can be concluded that the integrin glycans represent a high level of diversity playing different functions in integrin-mediated processes of adhesion and migration.

In conclusion, our studies show that the $\alpha_3\beta_1$ integrin from human ureter epithelium cell line HCV29 exhibits a highly heterogenous glycosylation pattern. The predominant oligosaccharides were of the bi- tri- and tetraantennary complex type. Some of these structures was fucosylated and sialylated. Also, the presence of short poly-*N*-acetyllactosamine moieties on both subunits but predominantly on β_1 was shown. The minor fraction represented high mannose residues.

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